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Intraerythrocytic inclusion bodies in the loggerhead sea turtle *Caretta caretta* from Madeira

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Intracytoplasmic inclusion bodies were detected in the mature red blood cells of twenty juvenile loggerhead sea turtles, *Caretta caretta*, captured in Madeira. The bodies were mostly single, round to oval, frequently irregular in outline, and their diameter varied from 0.5 to 2.0 μm. Most bodies were associated with small granular areas, often in the form of a tail or projection. In some cells, only granular areas were apparent. The nuclei of most erythrocytes were irregular in outline but degeneration of red blood cells was not observed. The identity of these intraerythrocytic structures is not clear but they may be viral or rickettsial in nature.

Small intraerythrocytic inclusion bodies have been described from the blood of numerous fishes, amphibians and reptiles (Davies & Johnston, 2000). Several found in fishes have been reported as examples of viral erythrocytic necrosis (VEN) (Eiras & Santos, 1992; Eiras et al., 1996), while those recorded in amphibia and reptiles have been assigned often to genera such as *Toddia* and *Pirhemocyton* (Acholonu, 1974; Paperna & Alves de Matos, 1993; Alves de Matos & Paperna, 1993). Transmission electron microscopy (TEM) has revealed that several VEN, *Pirhemocyton* and *Toddia* infections are associated with icosahedral DNA viruses, also known as iridoviruses (see Davies & Johnston, 2000). Another infection, found in the erythrocytes of water snakes (*Nerodia erythrogaster flavigaster*), has been attributed to oncornaviruses (Daly et al., 1980). Other inclusion bodies in the erythrocytes of ectotherms have been identified as rickettsial infections (see Davies & Johnston, 2000).

Information concerning the occurrence and identity of inclusion bodies within chelonian erythrocytes is scarce. Laveran (1903) regarded small, violet-staining structures in the cytoplasm of various chelonian red cells as particles eliminated from the nucleus, rather than haematozoa. Light microscopic studies by Acholonu (1974) led him to report *Pirhemocyton chelonarum* in five species of turtles from Louisiana, and similarly Peirce & Castleman (1974) described intraerythrocytic inclusions in the Moroccan tortoise (*Testudo graeca*), but they suggested that these parasites might be rickettsias. Intraerythrocytic parasites of turtles and tortoises, also suspected to be rickettsias, were listed by Barnard & Upton (1994), including one (*Grahamella thalassochelys*) from the loggerhead turtle, *Caretta caretta*. Recently, we were able to examine blood smears from specimens of *C. caretta*, from Madeira, Portugal, containing conspicuous intraerythrocytic inclusion bodies. The characteristics of the condition are reported in this paper.

Twenty specimens of juvenile *Caretta caretta* L. 1758 were dip netted at Madeira (3–10 nautical miles off Funchal) between April, 1995 and February, 1996. Carapace curved length varied from 22.2 to 67.5 cm (40.7 ±13.1 cm), and width between 21.4 and 65.3 cm (38.3 ±12.8 cm). Total weight ranged from 1.2 to 19.0 kg. The age of the specimens was estimated between 2–11 y using a von Bertalanffy equation derived from adult and subadult loggerhead turtles from the western Atlantic (Klinger & Musick, 1995). Blood was taken from the dorsal cervical sinus (Owens & Ruiz, 1980) using an heparinized syringe, and blood smears were stained with May–Grünwald–Giemsa.

In blood smears, 100% of the mature erythrocytes from all 20 turtles contained single, rarely two, intracytoplasmic inclusions, staining uniformly deep blue. These basophilic bodies were absent from erythroblasts. They were round to oval, frequently of irregular outline, and were between 0.5 and 2.0 μm in diameter. They occurred near the cell periphery, close to the nucleus, or at varying distances between the nucleus and the cell margin (Figure 1). No associated vacuoles or crystals were seen.

In most erythrocytes, inclusions were associated with small, irregular granular regions with poorly defined margins. Sometimes these regions occurred in the form of a tail or projection of the inclusion body. In other instances they existed at some distance from the inclusion body, or in its absence (Figure 1). Individual granules also stained blue, but often less intensely than the inclusion body itself; they measured no more than 0.25 μm in diameter and up to seven occurred within the granular region. A few infected macrocytes (giant erythrocytes) were present in blood smears but most infected erythrocytes were of normal size with nuclei that were of slightly irregular outline. Degeneration of the affected red blood cells was not observed, and therefore no pathological effects could be attributed to these inclusions.

The existence of an inclusion body measuring up to 2 μm across, often with a tail of granules, is frequently indicative of a viral infection within ectotherm erythrocytes. However, such inclusions usually stain pink with Giemsa, and may be associated with a vacuole (albuminoid body), as in several *Pirhemocyton* infections, or with a crystalline structure, as in many examples of *Toddia* (see Paperna & Alves de Matos, 1993). Viral erythrocytic necrosis inclusion bodies and related infections of fishes, also appear eosinophilic, but without vacuoles nor crystals. Conversely, oncornavirus infection reported by Daly et al. (1980) has vacuoles, but the inclusion bodies stain blue.

The structures seen in *C. caretta* also superficially resemble some bodies reported to be induced by rickettsias. We have not

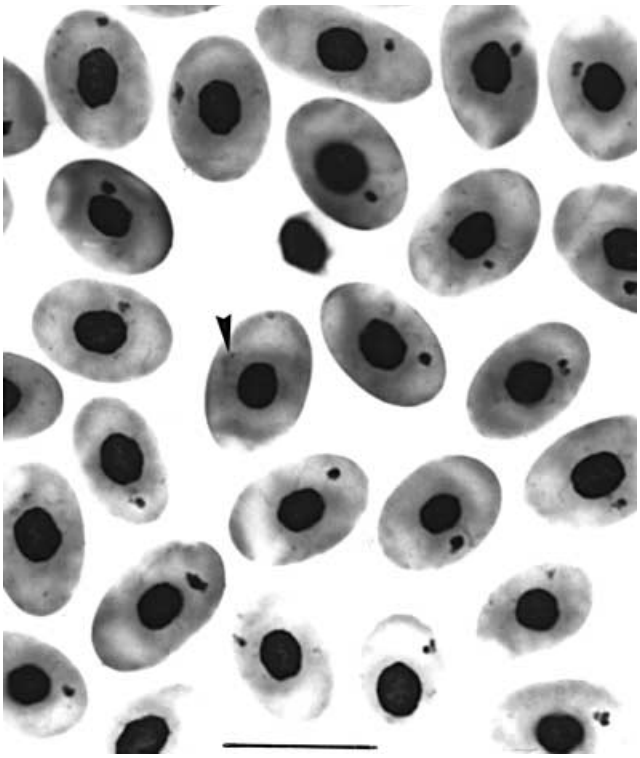

Figure 1. May–Grünwald–Giemsa stained blood smear from *Caretta caretta*. Note the inclusion bodies within the erythrocytes, and one cell showing only the presence of granular areas (arrow). Scale bar: 10 μm.

been able to trace the original description of *G. thalassochelys* from *C. caretta* by Cerruti (1931), but according to Weinman & Kreier (1977), *Grahamella* species stain purple–red with Giemsa, are bacilliform, and rarely coccoid. Our structures stained deep blue, were never bacilliform and they possessed granules. This suggests that they may not be a *Grahamella*. The structures from *C. caretta* in Madeira also resemble some stages of *Tunetella emydis* from the erythrocytes of *Emys leprosa* in Tunisia (Brumpt & Lavier, 1935). Barnard & Upton (1994) regarded *Tunetella* as a rickettsial infection, synonymous with *Aegyptianella*. However, basophilic bodies from the erythrocytes of the Moroccan tortoise, also attributed to rickettsias, (Peirce & Castleman, 1974) are much smaller than the inclusion bodies seen in *C. caretta*. No turtles without intraerythrocytic inclusions were found in this study, therefore it is difficult to predict how common these features are among loggerhead turtles generally. The decision whether the structures seen in *C. caretta* from Madeira are viral, rickettsial, or have another origin, awaits examination of the affected red cells by TEM.

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